Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/019631

International filing date: 17 June 2004 (17.06.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/478,835
Filing date: 17 June 2003 (17.06.2003)

Date of receipt at the International Bureau: 19 August 2004 (19.08.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1208312

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

August 06, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/478,835*
FILING DATE: *June 17, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/19631*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

60478835 .061703

PATENT

ATTORNEY DOCKET NO.: 057734-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PROVISIONAL APPLICATION FOR PATENT COVER SHEET

MAIL STOP PROVISIONAL PATENT APPLICATION
Commissioner for Patents
U.S. Patent and Trademark Office
2011 South Clark Place
Customer Window
Crystal Plaza Two, Lobby, Room 1B03
Arlington, VA 22202

This is a request for filing a PROVISIONAL APPLICATION under 37 C.F.R. § 1.53(c).

1. **INVENTOR(S):**

Last Name	First Name	MI	Residence
VIGH	Guyla		College Station, Texas
LALWANI	Sanjiv Kumar	S	College Station Texas

2. **TITLE: STABLE HIGH pI HYDROGEL COMPOSITIONS**

3. **APPLICATION PAPERS ENCLOSED:**
☒ Specification of 16 total pages
☒ Applicant(s) hereby assert small entity status under 37 C.F.R. §1.27.

4. **METHOD OF PAYMENT:** **FILING FEE:** $ 80.00
☒ The Commissioner is hereby authorized to charge the filing fee to Deposit Account No. 50-0310.
☒ The Commissioner is hereby authorized to charge any deficiency in the filing fee or credit any overpayment to Deposit Account No. 50-0310.

5. **CORRESPONDENCE ADDRESS:**
All correspondence shall be addressed to **Customer Number: 09629**

Respectfully Submitted:

MORGAN, LEWIS & BOCKIUS LLP

Erich E. Veitenheimer
Reg. No. 40,420

Date: June 17, 2003
1111 Pennsylvania Ave., N.W.
Washington, D.C. 20004
Telephone: (202) 739-3000
Facsimile: (202) 739-3001

STABLE HIGH pI HYDROGEL COMPOSITIONS

Technical Field

[0001] This invention relates to high-pI materials and hydrolytically stable, high-pI hydrogel and/or membrane compositions made from the materials. The primary application areas of the new hydrolytically stable, high-pI hydrogel and/or membrane compositions are in the analytical and preparative-scale isoelectric focusing separation and/or isoelectric trapping separation of ampholytic compounds.

Background

[0002] Electrophoretic techniques, and isoelectric focusing (IEF) techniques in particular, remain key technologies for the separation of ampholytic components, small and large, simple and complex alike. Used in many fields and industries, IEF is performed both on an analytical and preparative scale. For example, IEF is utilized in clinical diagnosis, biotechnology, pharmaceutical and food industries, etc., alone or coupled with other analytical or preparative techniques.

[0003] In IEF, ampholytic components are separated with the help of an electric field in a pH gradient wherein the pH increases from a lower pH value at the anode to a higher pH value at the cathode. (For a monograph on IEF, see, e.g., P.G. Righetti, Isoclectric focusing: theory, methodology and applications, Elsevier Biomedical, Amsterdam, 1983, which is herein incorporated by reference). As the net charge of an ampholytic component is zero in its isoelectric state, the electrophoretic migration velocity of an ampholytic component becomes zero whenever the pH of its environment becomes equal to its isoelectric point (pI) value. Thus, ampholytic components with different pI values stop migrating at different points in the pH gradient.

[0004] Relatively stable continuous pH gradients can be created by several means. For example, mixtures of carrier ampholytes (compounds that have adequate buffering ability and conductivity in the vicinity of their pI value) may be used. Also, appropriate amounts of suitable weak acids and weak bases or weak acids and strong bases or strong acids and weak bases may be bound, in a spatially controlled manner, into an ion-permeable matrix,

such as a cross-linked polyacrylamide gel to preform and stabilize the pH gradient which is then used for immobilized pH gradient IEF (IPGIEF). (For a monograph on IPGIEF, see, e.g., P.G. Righetti, Immobilized pH gradients: theory and methodology, Elsevier, Amsterdam, 1990, which is herein incorporated by reference.).

[0005] Alternatively, ampholytic sample components can also be separated from each other by isoelectric trapping (IET) utilizing isoelectric membrane-based multicompartmental electrolyzers (e.g., Faupel et al., U.S. Patent No. 5,082,548, which is incorporated herein by reference) wherein at the end of an IET separation process, ampholytic sample components are obtained in their isoelectric state.

[0006] Unfortunately, the present IEF or IET technologies are not particularly suitable for the separation or processing of compounds having very high pI values as the separation media presently employed are not particularly stable at these extreme pH values. Thus, there is a real need for hydrolytically stable, high-pI hydrogel and/or membrane compositions.

[0007] The present inventors have now developed hydrolytically stable, high-pI hydrogel and/or membrane compositions.

Summary of Invention

[0008] In a first aspect, the present invention provides a high-pI isoelectric hydrogel material comprising a carbohydrate-based compound or a polyhydroxy compound having one or more secondary OH groups with a pK_a value between about 10.5 and 14 reacted with an agent having a strongly basic functional group and a crosslinking agent to form a material having a pI value greater than about 10.5.

[0009] The carbohydrate-based compound may also contain at least one primary OH group with a pK_a value greater than about 13.

[0010] The carbohydrate-based compound or polyhydroxy compound can be monosaccharides, disaccharides, trisaccharides, oligosaccharides and polysaccharides, including native or partially hydrolyzed cyclodextrins, maltodextrins, amyloses, starches, dextrans, celluloses, luteoses, curdlans, guar gums, agaroses, and the like. It will be

appreciated that other compounds with pKa values in the 10.5 to 14 range would also be suitable for the present invention.

[0011] Preferably, the basic functional group is a quaternary ammonium group. Examples of suitable quaternary ammonium groups include, but not limited to, trimethylammonium, triethylammonium, tripropylammonium, tributylammonium, dimethylethylammonium, methyldicthylammonium, other trialkylammonium groups containing identical or different alkyl groups, tri(hydroxyalkyl)ammonium groups, dialkyl(hydroxyalkyl) ammonium groups, alkyl(dihydroxyalkyl)ammonium groups, N-morpholinium groups, N-piperidinium groups, N-pyrrolidinium groups, N-quinuclidinium groups, N-pyridinium groups. It will be appreciated that there would be other quaternary ammonium groups suitable for use in the present invention.

[0012] Preferably, the crosslinking agent is a difunctional, trifunctional or multifunctional agent capable of reacting with the primary or secondary alcohol group-containing moity, such as dialdehydes, including but not limited to, glutaraldehyde, diepoxydes, including but not limited to glycerol-1,3-diglycidyl ether, dihalides, including but not limited to bis(bromoethylene)ethyleneglycol, ditosylates, including but not limited to bis(tosylethylene)ethyleneglycol. It will be appreciated that there would be other crosslinking agents suitable for use in the present invention.

[0013] The isoelectric hydrogel material according to the present invention preferably has a pI of greater than about 11. The hydrogel materials can be prepared having a pI from about 10.5 to about 14.

[0014] In a second aspect, the present invention provides a hydrolytically stable high-pI hydrogel membrane comprising a high-pI isoelectric material according to the first aspect of the present invention supported on an inert or crosslinkable or crosslinked substrate.

[0015] It will be appreciated that the substrate may be any suitable material capable of supporting a hydrogel to form a membrane. Preferably, the substrate is selected from, but not limited to, polyvinylalcohol, partially or fully hydrolysed poly(epihalohydrine), partially or fully hydrolysed poly(epihalohyrine-co-polyethylene oxide), polyvinylsulfone, polyether ether ketone. It will be appreciated that there would be other substrates suitable for use in the present invention.

[0016] In a third aspect, the present invention provides a method for forming a high-pI isoelectric hydrogel material comprising:

reacting a carbohydrate-based compound having one or more secondary OH groups with a pK_a value between about 10.5 and 14 with an agent having a strongly basic functional group to form a material having a pI value greater than about 11 and crosslinking it to form a hydrogel.

[0017] In a fourth aspect, the present invention provides a method for forming a hydrolytically stable high-pI hydrogel membrane comprising:

carrying out the method according to the third aspect of the present invention; and

applying the high-pI isoelectric material onto an inert or crosslinkable or crosslinked supporting substrate to form a hydrolytically stable high-pI hydrogel membrane having a pI value greater than about 10.5.

[0018] In a fifth aspect, the present invention provides a high-pI isoelectric hydrogel material produced by the method according to the third aspect of the present invention.

[0019] In a sixth aspect, the present invention provides a high-pI isoelectric hydrogel material membrane produced by the method according to the fourth aspect of the present invention.

[0020] In a seventh aspect, the present invention provides use of hydrolytically stable high-pI hydrogel membrane having a pI value greater than about 10.5 according to the present invention in the separation of an ampholytic compound by electrophoresis.

[0021] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0022] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the

field relevant to the present invention as it existed before the priority date of the invention.

[0023] In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following examples.

Mode(s) for Carrying Out the Invention

[0024] In membrane-mediated isoelectric focusing and isoelectric trapping separations it would often be desirable to utilize hydrogels and/or membranes that have pI values above 10.5. These isoelectric hydrogels and/or membranes can serve either as ion-permeable separative barriers or ion-permeable cathodic electrode compartment barriers. The isoelectric hydrogels and/or membranes can play multiple roles to effect or aid the desired separations by their buffering and/or sieving ability and prevent convective mixing. Typically, the ion-permeable barriers, hydrogels and/or membranes are prepared from acrylamide and/or acrylamido weak and/or strong electrolyte derivatives by polymerizing suitable monomers, modifiers and crosslinkers. Though acrylamide-based ion-permeable barriers, hydrogels and/or membranes have many outstanding and desirable characteristics, neither the monomers, nor the polymers are hydrolytically stable at above about pH 10.5. Hydrolysis of the amide bond creates weak acid functional groups in the polyacrylamide-based matrix which, upon dissociation, act as immobilized negative charges in the gel matrix, create electroosmotic flow and/or bind certain analytes, both of which are often detrimental to the separation.

[0025] In addition to the limited hydrolytic stability of the acrylamide-based hydrogels and/or membranes in high pH environments, the formation of pI >10.5 isoelectric hydrogels and/or membranes is hindered by the lack of suitable acrylamido weak base derivatives with pK_b values between 2 and 4.

[0026] Our work in the field of cyclodextrins taught us that the pK_a values of the secondary alcohol groups of certain carbohydrates and polyhydroxy compounds lie in the $10.5 < pK_a$ range, while the pK_a values of the primary alcohol groups are typically in the $pK_a > 13$ range. For example, the pKa values a few common carbohydrates are as follows (Bruggink, C., AVH Association - 7th Symposium - Reims, March 2000 3-9;

Masuda, T., et al., *J. Chromatogr A.*, 961 (1) 89-96; Lee, Y-H. and Lin, T., *Electrophoresis*, 17, 333-340; Rong, D. and D'Souza, V., *Tetrahedron Letters*, 31 (30) 4275-4278, all incorporated herein by reference):

Compound	**pK_a**
Maltose	11.94
Lactose	11.98
Fructose	12.03
Mannose	12.08
Xylose	12.15
Glucose	12.28
Galactose	12.39
Dulcitol	13.43
Sorbitol	13.60
α-Methyl glucoside	13.71
1,3-dimethoxy glycerol	13.68
γ-cyclodextrin	12.05 (secondary OH)
β-cyclodextrin	12.20 (secondary OH)
α-cyclodextrin	12.33 (secondary OH)

[0027] Thus, if one could create a molecule that would contain at least one such secondary OH group with a $10.5 < pK_a < 14$ and an amine group with a $1 < pK_b < 4$ or at least two such secondary OH groups with pK_a values in the $10.5 < pK_a < 14$ range and one strongly basic functional group (such as a quaternary ammonium group) or at least one such secondary OH group with a pK_a value in the $10.5 < pK_a < 14$ range and one such primary OH group with a pK_a value in the $13 < pK_a$ range and one permanently cationic functional group (such as a quaternary ammonium group), one would be able to create an isoelectric substance with a high pI value. Once such an isoelectric material was created, it could be turned into a hydrogel and/or membrane by crosslinking it with any suitable bifunctional or polyfunctional agent or by grafting it onto any suitable crosslinkable or crosslinked substrate.

[0028] We have also recognized that the secondary alcohols of many oligo- and polysaccharides including, but not restricted to, cyclodextrins, maltodextrins, amyloses, starches, dextrans, celluloses, luteoses, curdlans, guar gums, agaroses, etc., have the same desired property (secondary OH groups with pK_a values in the $10.5 < pK_a < 14$ range, primary OH groups in the $13 < pK_a$ range) and these oligomers and/or polymers can be modified with suitable amine or quaternary ammonium functional groups to create high pI isoelectric materials which can be converted, e.g., by crosslinking, into suitable hydrogels and/or membranes.

[0029] Furthermore, we have recognized that the secondary alcohol groups of many oligomeric and polymeric materials including, but not restricted to, poly(vinylalcohol) and its derivatives, partially or fully hydrolyzed poly(epihalohydrine)s and their derivatives, partially or fully hydrolyzed poly(epihalohydrine-co-ethylene oxide)s and their derivatives polymers formed from polyhydroxy compounds and di-, oligo- or polyepoxides also have the same desired property (secondary OH groups with pK_a values in the $10.5 < pK_a < 14$ range and primary OH groups with pK_a values in the $13 < pK_a$ range) and these oligomers and/or polymers can be modified with suitable amino or quaternary ammonium functional groups to create high-pI isoelectric materials which can be converted, e.g., by crosslinking, into suitable hydrogels and/or membranes.

[0030] Finally, we have recognized that the hydrophilic, polymeric nature of such hydrogels and/or membranes reduces the magnitude of electroosmotic flow through such hydrogels and/or membranes, which is very desirable for electrophoretic separations.

[0031] By varying the concentration of the OH group-containing material and/or the type and/or the concentration of the crosslinking agent and the cationic functional group, the present invention can produce high-pI isoelectric hydrogels and/or membranes that can also act as sieving matrices in electrophoretic separations, similarly to the way acrylamide-based gels do.

[0032] It will be appreciated that many additional tasks can be solved utilizing the hydrolytically stable, high-pI isoelectric hydrogel and/or compositions according to the present invention without departing from the essence of this disclosure.

APPARATUS

[0033] A membrane-based electrophoresis apparatus particularly suitable for isoelectric focussing or isoelectric trapping has been developed by The Texas A&M University System and Gradipore Limited (WO 02/24314, which is incorporated herein by reference). The apparatus, termed herein as "the Twinflow unit" comprises (a) a first electrolyte reservoir and a second electrolyte reservoir; (b) a first sample reservoir and a second sample reservoir; (c) a separation unit having a first electrolyte chamber in fluid connection with the first electrolyte reservoir, a second electrolyte chamber in fluid connection with the second electrolyte reservoir, a first sample chamber positioned between the first electrolyte chamber and the second electrolyte chamber, a second sample chamber positioned adjacent to the first sample chamber and between the first electrolyte chamber and the second electrolyte chamber, the first sample chamber being in fluid connection with the first sample reservoir, and the second sample chamber being in fluid connection with the second sample reservoir; (d) a first ion-permeable barrier positioned between the first sample chamber and the second sample chamber, the first ion-permeable barrier prevents substantial convective mixing of contents of the first and second sample chambers; (e) a second ion-permeable barrier positioned between the first electrolyte chamber and the first sample chamber, the second ion-permeable barrier prevents substantial convective mixing of contents of the first electrolyte chamber and the first sample chamber; (f) a third ion-permeable barrier positioned between the second sample chamber and the second electrolyte chamber, the third ion-permeable barrier prevents substantial convective mixing of contents of the second electrolyte chamber and the second sample chamber; (g) electrodes positioned in the first and second electrolyte chambers; (h) means for supplying electrolyte from the first electrolyte reservoir to the first electrolyte chamber, and from the second electrolyte reservoir to the second electrolyte chamber; and (i) means for supplying sample or liquid from at least the first sample reservoir to the first sample chamber, or from the second sample reservoir to the second sample chamber.

[0034] In use, a sample to be treated is placed in the first and/or second sample reservoirs and provided to, or circulated through, the first and/or second chambers.

Electrolyte is placed in the first and second electrolyte reservoirs and passed to, or circulated through, the respective first and second electrolyte chambers without causing substantial mixing between the electrolyte in the two electrolyte reservoirs. Electrolyte or other liquid can be placed in first and/or second sample reservoirs if required. An electric potential is applied to the electrodes wherein one or more components in the first and/or second sample chamber are caused to move through a diffusion barrier to the second and/or first sample chamber, or to the first and/or second reservoir chambers. Treated sample or product can be collected in the second and/or first sample reservoir.

METHODS

[0035] In developing the present invention, various hydrogels were prepared at temperatures ranging from room temperature to about 80°C and reaction times varying from a few minutes to several days. Higher temperatures were used in order for the reaction to proceed at a reasonable rate. It has been found, however, that the actual reaction temperature and time of reaction incubation are not particularly critical to develop various hydrogels according to the present invention. It will be appreciated that as temperatures are elevated, the rate of reaction will increase si incubation times will be shorter. Accordingly, reaction conditions can be selected in order to determine how long the reaction needs to proceed.

[0036] Preferred methods for producing hydrogels and membranes according to the present invention are set out below. It will be appreciated, however, that it is within the skill of the art to alter conditions for any given hydrogel preparation.

[0037] Weigh a 100 mL beaker. Place the weighed 100 mL beaker and two 230 x 190 x 6 mm, clean glass plates into a drying oven at 80°C. Cut a 160 x 200 mm piece of a Grade BFN 3 Papylon PVA paper (Sansho Co., Ltd, The 2nd Kitahama Building 1-29, Kitaham-Higashi, Chuoh-Ku, Osaka, Japan). Fit a 250 mL, two-neck, round bottom flask with a condenser and a nitrogen purge line. Place a 1" football-shaped stir bar into the flask. Purge the system with nitrogen gas. Circulate ice-water through the condenser.

[0038] Place the flask into a heating mantle. Put on protective gloves. Add 60 mL deionized water to the flask. Add 6.58 g (0.1645 mol) NaOH to the flask. Begin stirring and heat the solution to a boil. Add 12 g (0.2727 mol secondary OH group equivalent)

99% hydrolyzed poly(vinylalcohol), average molecular weight 89,000 - 98,000 (PVA) to the flask. Maintain a nitrogen atmosphere over the reaction mixture, continue stirring and heating until PVA is completely dissolved. Turn off the heating mantle. Add 1.8 g (0.012 mol) glycidyl trimethylammonium chloride (Q) to the reaction mixture and stir until Q is dissolved.

[0039] Take the hot, bottom glass plate from the oven and place it onto a layer of paper towels. Take the hot, 100 mL beaker from the oven and weigh into it a 60 g aliquot of the hot, viscous reaction mixture. Quickly add to it 4.5 mL (4.916 g, 0.024 mol) glycerol diglycidyl ether and mix it well (manually) with a spatula. Pour half of the beaker's content onto the hot, bottom glass plate and quickly distribute the mixture over the plate by tilting it around. Lower the BFN 3 PVA substrate onto the reaction mixture and saturate the substrate with the reaction mixture.

[0040] Take the hot, cover glass plate from the oven, pour the second half of the reaction mixture from the beaker onto it and quickly distribute the mixture over the plate by tilting it. Lower the coated face of the cover plate onto the BFN 3 PVA substrate and press the plate to evenly distribute the reaction mixture over the entire surface of the BFN 3 PVA substrate. Place two 16 x 16 x 2" cement patio paving stones onto the glass plates to compress them and squeeze out the excess reaction mixture.

[0041] Two hours later, remove the stones from the glass plates. Let the glass plate mold stand undisturbed at room temperature for 38 hours (total curing time 40 hours).

[0042] Fill a 16 x 12 x 6" polypropylene tub with deionized water. Using a razor blade, cut along all four edges of the glass plate mold to remove the solidified, spilled-out reaction mixture. Lower the mold into the deionized water in the tub. Gently pull the glass plates apart under the water. The membrane should slip off easily from the glass plates. Gently slosh around the membrane in the water for about five minutes. Replace the water, slosh around the membrane for another five minutes. Repeat the procedure at least five times. Test the pH of the last wash water, it should be neutral. The salvage edge of the membrane should be clear, transparent, the surface of the membrane strong, even and slippery.

[0043] Store the membrane in deionized water in the fridge until used. The membrane will swell to a final thickness of about 0.4 to 0.7 mm. Residual reactivity of the membrane due to left-over epoxide groups is unknown, so handle it with care, wearing gloves. Using a pair of scissors, cut the membrane to size to fit the separation cartridge of the Twinflow unit. Punch inlet and outlet holes into the membrane and assemble the cartridge. Leak test the Twinflow unit, then commence the separation. After use, rinse the membrane and dispose it as solid waste.

[0044] The new compositions permit the preparation of hydrolytically and mechanically stable, high-pI hydrogels and/or membranes that were not available prior to this invention.

[0045] Numerous other hydrophilic, hydrolytically stable, high-pI compositions can be created along the synthetic lines described above, and these are expected to work just as well as the examples described below.

EXPERIMENTAL

[0046] The feasibility of creating hydrolytically stable high-pI hydrogels and/or membranes as outlined above has been experimentally demonstrated as follows.

Example 1

[0047] High-pI, clear hydrogels were prepared by crosslinking trimethylammonio-β-cyclodextrin with epichlorohydrin in the presence of NaOH, at 80°C.

Example 2

[0048] High-pI, clear hydrogels were prepared by crosslinking trimethylammonio-β-cyclodextrin with glycerol diglycidyl ether in the presence of NaOH, at 80°C.

Example 3

[0049] High-pI, clear hydrogels were prepared by crosslinking trimethylammonio-β-cyclodextrin and poly(vinylalcohol) with glycerol diglycidyl ether in the presence of NaOH, at 80°C.

Example 4

[0050] High-pI, clear hydrogels were prepared by crosslinking β-cyclodextrin and poly(vinylalcohol) with glycerol diglycidyl ether in the presence of glycidyl trimethylammonium chloride and NaOH, at 80°C.

Example 5

[0051] High-pI isoelectric membranes were prepared by casting, in a glass mold, an 80°C reaction mixture of glycidyl trimethylammonium chloride, β-cyclodextrin, poly(vinylalcohol), glycerol diglycidyl ether and NaOH over a Papylon Grade 3 poly(vinylalcohol) substrate and reacting the mixture at 60°C for 40 hours.

Example 6

[0052] High-pI isoelectric membranes prepared in Example 5 above were tested in the Twinflow unit described above. The membranes were used in a single separation compartment configuration, as the cathodic membrane. The anodic membrane was a pI = 3 polyacrylamide isoelectric membrane (Gradipore Limited, Australia). The anolyte was 50 mM benzenesulfonic acid (BSH), the catholyte 50 mM benzyltrimethylammonium hydroxyde (BzOH)and 950 mM NaOH, the separation compartment contained tyramine (Tyr, approximate pI = 10), histidine (His, pI = 7.5) and meta-aminobenzoic acid (MABA, approximate pI = 3.9) as analytes. Leak-free seal was achieved, and MABA, His and Tyr were trapped for the duration of the 180 min run. Neither BSH, nor BzOH invaded the separation compartment. When the run was repeated with a 50 mM benzyltrimethylammonium hydroxyde solution as the catholyte, Tyr was lost to the cathode compartment within 15 min indicating that the pI value of the high-pI membrane was greater than 12.7.

Example 7

[0053] High-pI, clear hydrogels were prepared by reacting trimethylammonio-guar gum with glycidyl trimethylammonium chloride and crosslinking it with glycerol diglycidyl ether in the presence of NaOH, at 80°C.

Example 8

[0054] High-pI, clear hydrogels were prepared by reacting glycidyl trimethylammonium chloride with poly(vinylalcohol), and crosslinking it with glycerol diglycidyl ether, in the presence of NaOH, at 80°C.

Example 9

[0055] High-pI isoelectric membranes were prepared by casting, in a glass mold, an 80°C reaction mixture of glycidyl trimethylammonium chloride, poly(vinylalcohol), glycerol diglycidyl ether and NaOH over a Papylon Grade 3 poly(vinylalcohol) substrate and reacting the mixture at 60°C for 24 hours.

Example 10

[0056] The high-pI isoelectric membranes prepared in Example 9 above were successfully tested in the Twinflow unit, in single separation compartment configuration, as the cathodic membrane. The separation compartment contained Tyr (pI = 10), His (pI = 7.5) and MABA (pI = 3.9) as analytes. Leak-free seal was achieved and MABA, His and Tyr were trapped for the duration of the 180 min run. When the run was repeated with a pH 12 NaOH solution as the catholyte, Tyr was lost to the cathode compartment, within 15 min, indicating that the pI value of the high pI membrane was greater than 12.

Example 11

[0057] High-pI isoelectric membranes were prepared by casting, in a glass mold, an 80°C reaction mixture of glycidyl trimethylammonium chloride, poly(vinylalcohol), glycerol diglycidyl ether and NaOH over a Papylon Grade 3 poly(vinylalcohol) substrate and reacting the mixture at room temperature for 40 hours.

Example 12

[0058] The high-pI isoelectric membranes prepared in Example 11 above were successfully tested in the Twinflow unit, in single separation compartment configuration,

as the cathodic membrane. The separation compartment contained Tyr (pI = 10), His (pI = 7.5) and MABA (pI = 3.9) as analytes. Leak-free seal was achieved and MABA, His and Tyr were trapped for the duration of the 180 min run.

Example 13

[0059] High-pI isoelectric membranes were prepared by casting, in a glass mold, an 80°C reaction mixture of glycidyl trimethylammonium chloride, poly(vinylalcohol), glycerol diglycidyl ether and NaOH over a Papylon Grade 4 poly(vinylalcohol) substrate and reacting the mixture at room temperature for 40 hours.

Example 14

[0060] High-pI isoelectric membranes prepared in Example 13 above were successfully tested in the Twinflow unit, in single separation compartment configuration, as the cathodic membrane. The separation compartment contained Tyr (pI = 10) and MABA (pI = 3.9) as analytes. Leak-free seal was achieved and both MABA and Tyr were trapped for the duration of the 180 min run.

Example 15

[0061] High-pI isoelectric membranes were prepared by casting, in a glass mold, an 80°C reaction mixture of glycidyl trimethylammonium chloride, poly(vinylalcohol), glycerol diglycidyl ether and NaOH over a Papylon Grade 2 poly(vinylalcohol) substrate and reacting the mixture at room temperature for 40 hours.

Example 16

[0062] High-pI isoelectric membranes prepared in Example 15 above were successfully tested in the Twinflow unit, in single separation compartment configuration, as the cathodic membrane. The separation compartment contained Tyr (pI = 10), His (pI = 7.5) and MABA (pI = 3.9) as analytes. Leak-free seal was achieved and both MABA and Tyr were trapped for the duration of the 180 min run.

Example 17

[0063] High-pI isoelectric membranes prepared in Example 15 and Example 16 above were tested in the Twinflow unit, in single separation compartment configuration, as the cathodic membranes. The separation compartment contained a recombinant thyroid-stimulating hormone (rTSH) preparation in a growth medium with a conductivity of 5000 μS. Desalting of the sample was successfully completed to a residual conductivity of about 800 μS, and neither albumin, nor rTSH was lost indicating that the high-pI isoelectric membranes functioned properly as cathodic isoelectric membranes.

Example 18

[0064] High-pI isoelectric membranes prepared according to Example 11 and Example 15 above were successfully tested as cathodic membranes in over 10 isoelectric trapping (IET) separations using the Twinflow unit. Each time, when the NaOH concentration in the catholyte was 200 mM or higher, and the IET current was sufficiently high, the membranes behaved satisfactorily.

[0065] It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

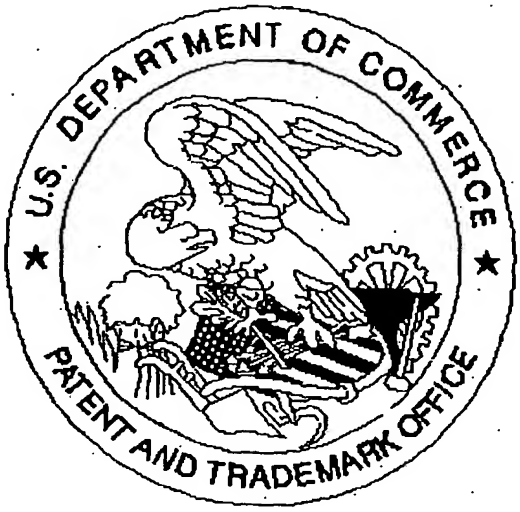
Abstract

A high-pI isoelectric hydrogel material comprising a carbohydrate-based compound or a polyhydroxy compound having one or more secondary OH groups with a pK_a value between about 10.5 and 14 reacted with an agent having a strongly basic functional group to form a material having a pI value greater than about 10.5 and crosslinked to form a hydrogel.

60478835 .061703

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

☐ Page(s)_____ of Small entity status were not present for scanning.
(Document title)

☐ Page(s)_____ of_____ were not present for scanning.
(Document title)

☐ ***Scanned copy is best available.***